On page 6, line 23, after the word "myocytes.", please insert -- **(A)** Myoblasts infected with Adeno-Akt express the Akt transgene as indicated by Western immunoblot analysis using either anti-Akt or anti-HA antibodies. **(B)** Anti-Akt and anti-HA immunoprecipitates from Adeno-Akt-infected myoblast cultures also contained appreciable levels of H2B kinase activity, indicating that the transgene produces functional Akt protein. **(C)** Cultures infected with Adeno-Akt displayed significantly less cell-death than control cultures. --

On page 6, line 27, after the phrase "mean +/- S. E. M.", please insert -- Control cultures (closed circles) received vehicle alone. were infected with an adenoviral vector expressing β-galactosidase (Ad- β-gal), which does not affect endothelial cell viability under the conditions of our assay. As shown in Fig. 3B, adenoviral transfection of Akt markedly augmented VEGF-induced endothelial cell survival. In brief, HUVECs were cultured in 24 wells (Falcon) and infected with adenoviral vector expressing Akt (Ad-Akt) or β-galactosidase (Ad- β-gal) at a MOI of 50. After 24 hour incubation, the medium was changed to DMEM containing the indicated concentrations of VEGF. After 21 hours culture, viable cells were counted. Figure 3 Inset: HUVEC cultures were infected with Ad- β-gal (β gal) or Ad-Akt (Akt) and incubated for 24 hours. After 30 min. serum starvation, cells were treated with 1 ng/ml of VEGF for 15 min. The cell lysates were prepared and immunoprecipitated with anti-Akt antibodies. The kinase activities were measured as described in Materials and Methods. Cultures infected with Ad-Akt displayed 75 and 65% less cell-death than control cultures at 1 and 10 ng/ml, respectively, while no decrease in cell-death was detected in the cultures exposed to serum-free media in the absence of VEGF. As anticipated, adenoviral transfection of Akt also enhanced Akt kinase activity (Fig. 3B, inset). Akt immunoprecipitates prepared from Ad-Akt -infected HUVEC cultures exhibited greater kinase activity than control cultures when exposed to 1 ng/ml VEGF, the concentration of factor that produced the greatest difference in survival between test conditions. These data show that forced Akt expression can enhance the sensitivity of endothelial cells to VEGF survival signals.--

Remarks:

The specification page 1 is amended herewith to reflect the priority status of the present application.

The figure description on page 6 is amended herewith to include a more detailed description of Figures 2A, 2B, 2C, and 3B.

No new matter has been added.

Information Disclosure Statement:

The Examiner states that Applicants previously did not enclose a Modified PTO-1449 form or the International Search Report in PCT/US99/22633 that was identified in the Information Disclosure Statement. Applicants submit herewith copies of the requested documents. Applicants respectfully request that the Examiner consider these documents and indicate that he has done so on the Modified PTO-1449 form previously submitted.

Specification:

The Examiner has requested correction to the specification on page 1 to reflect the priority status of the present application. The Examiner also has requested that the Figure description on page 6 be amended to include a further description of Figures 2A, 2b, 2C, and 3B.

The specification pages 1 and 6 are amended herewith in accordance with the Examiner's Request. In particular, with respect to the Figure description amendment, Applicants have inserted into the description on page 6 the more complete description of the figures as originally provided on pages 39-40 (Figure 2A, 2B, and 2C) and on page 43 (Figure 3B, including inset). The description of Figure 3B states that the adenoviral transfection of Akt marketly augmented VEGF-induced endothelial cell survival. Endothelial cell survival is indicated in the Y axis of Figure 3B (relative cell number) as a function of the VEGF concentration. Accordingly, the upper line (open circles) which shows enhanced VEGF-induced endothelial cell represent the experimental results obtained in the presence an adenoviral vector expressing Akt (Ad-Akt) and the lower line (closed circles) represents the results obtained in the presence of a control vector, namely, an adenoviral vector expressing β-galactosidase (Ad-β-gal). The inset in Figure 3B illustrates the enhanced Akt kinase activity resulting for Akt immunoprecipitates prepared from Ad-Akt-infected cultures (inset right-hand side) compared to control cultures infected with Ad-β-gal (inset left-hand side) when exposed to 1 mg/ml VEGF.

In view of the foregoing explanation and amendment, Applicants respectfully request that the Examiner reconsider and withdraw the objections to the specification on pages 1 and 6.

Rejection of Claims 1-4 under 35 U.S.C. §103(a):

Claims 1-4 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Cuevas et al. (Eur. J. Med. Res., Vol. 2, pages 465-468, November, 1997) in view of Datta et al. (Cell, Vol. 91, pages 231-241, October, 1997). The Examiner states that the claims are directed to a method for treating myocardial infarction comprising administering to a subject an Akt molecule in an amount effective to inhibit cardiac tissue necrosis (Claim 1). The dependent claims further specify that the cardiac tissue necrosis is mediated by increased apoptotic cell-death of a cardiomyocyte (Claim 2) wherein the cardiac tissue necrosis is mediated by increased apoptotic cell-death of a cardiac tissue endothelial cell (Claim 3) and wherein the Akt molecule is administered acutely (Claim 4). According to the Examiner, Cuevas et al. teach a method for treating myocardial infarction that involves "administering to a subject in need of such treatment a molecule in an amount effective to inhibit cardiac tissue necrosis (abstract) wherein the cardiac tissue necrosis is mediated by increased apoptotic cell-death of a cardiomyocyte (introduction and page 466, 1st column) and a cardiac tissue endothelial cell (Fig. 1, page 467) wherein the molecule is also administered acutely (abstract). The Examiner acknowledges that the Cuevas et al. reference does not disclose an Akt molecule in the treatment protocol; however, the Examiner further states that the Datta et. al. reference teaches that the Akt molecule is an inhibitor of apoptosis in "a variety of cell types (page 231, 2nd column, 2nd full paragraph)." The Examiner concludes that it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to "substitute the apoptotic inhibitor used by Cuevas et al. with the Akt molecule taught by Datta et al. in order to treat a myocardial infarction because both molecules are well known in the art to function as inhibitors of apoptosis." The Examiner further states that based on the Datta et al. teachings, one skilled in the art would have expected that an Akt molecule would also treat a myocardial infarction. The Examiner relies on the Cuevas et al. reference for motivation to make this substitution because Cuevas et al. "successfully teach that when FGF was given as a systemic bolus immediately after myocardial ischemia, apoptosis was significantly reduced by 60%." In conclusion, the Examiner states that "since both molecules function to inhibit apoptosis, it would have been obvious to one of ordinary skill in the art to use an Akt molecule to treat myocardial infarctions."

Applicants respectfully traverse this rejection for the reasons set forth below.

In general, Applicants agree with the Examiner's characterization of the claimed invention and of the references. However, Applicants traverse the Examiner's conclusion that one of ordinary skill in the art would have been motivated to substitute the Akt molecule as taught by Datta et al. for the FGF molecule as taught by Cuevas et al. to result in the invention as claimed. The Examiner correctly acknowledges that the Cuevas et al. reference does not teach or suggest the use of an Akt molecule for treating myocardial infarction. The only purported nexus between the Cuevas et al. and the Datta et al. references is that each relates, in a *general* sense to an apoptotic process. The Examiner relies upon the Datta et al. reference for showing that an "Akt molecule is an inhibitor of apoptosis in a variety of cell types." This reliance is misplaced. Datta et al. does not teach, suggest, or render obvious that Akt would be expressed in the types of cells which play a critical role in myocardial infarction. The paragraph cited by the Examiner for support that Datta teaches an Akt molecule as an inhibitor of apoptosis in a variety of cell types is quoted below in its entirety. (page 231, second column, second full paragraph):

> Akt is a general mediator of growth factor-induced survival and has been shown to suppress the apoptotic death of a number of cell types induced by a variety of stimuli, including growth factor withdrawal, cell-cycle discordance, loss of cell adhesion, and DNA damage (Ahmed et al., 1997; Dudek et al., 1997; Kauffmann-Zeh et al., 1997; Kennedy et al., 1997; Khwaja et al., 1997; Kulik et al., 1997). Thus, a signaling pathway has been defined in which growth factor receptor activation leads to the sequential activation of PI3'K and Akt, which then, through as-yet undescribed mechanisms, promotes cell survival and blocks apoptosis.

None of the references cited in the above-quoted paragraph teach or suggest the expression of Akt in the cell types which are the subject of the pending application. As stated in the application as filed on page 2 (emphasis added),

> "... Activation of Akt reportedly inhibits apoptosis induced by growth factor withdrawal or irradiation in neural cells, fibroblasts, and lymphocytes (Franke, T.F., et al., *Science*, 1997, 275:665-668; Hemmings, *Science*, 1997, 275:628-630)...."

"The invention involves the discovery that Akt ... inhibits apoptotic cell-death of cardiomyocytes, skeletal myocytes and/or vascular endothelial cells. In view of these discoveries, it is believed that Akt molecules can be used to inhibit apoptotic cell-death of the afore-mentioned cell types, and in particular, to treat conditions (e.g., myocardial infarction) that result in increased apoptotic cell-death of cardiomyocytes, skeletal myocytes and/or vascular endothelial cells."

The expression of Akt in one cell type, would not lead one of ordinary skill in the art to have a reasonable expectation that Akt would be expressed in a completely different cell type and in response to different and more complex stimuli, e.g. ischemia and ischemia-reperfusion injury which involves the accumulation of metabolic waste products, changes in mechanical factors and the generation of toxic substances. Accordingly, Applicants' discovery of the expression of Akt in cardiomyocytes, skeletal myocytes and vascular endothelial cells and the use of this discovery to treat conditions that are mediated by expression of Akt in these cell types is neither taught nor suggested by the prior art teachings that Akt is expressed in neural cells, fibroblasts, and lymphocytes. In contrast to the cited art, each of the pending claims is directed to targeting Akt expression in the specific cell types which were the subject of Applicants' discovery.

Applicant respectfully traverses the basis for this rejection for the following additional reasons:

The Examiner's burden that has to be met for a proper and legal rejection based on a combination of references has been explained by the Court of Appeals for the Federal Circuit (CAFC) (In re Dembiczak, 50 USPQ2d 1614, Fed. Cir. 1999). As explained below, this burden is a heavy one; however, no valid rejection can be made over a combination of prior art without satisfying this burden.

In Dembiczak, the CAFC held that the Patent Office failed to establish a case of prima facie obviousness because there was no motivation in the art to combine the teachings of references in the manner suggested by the Patent Office examiner without applying hindsight. According to the Dembiczak court (citations omitted):

"35 U.S.C. 103 bars patentability when the invention would have been obvious "at the time that the invention was made. ... [I]t is this phrase that guards

against entry into the 'tempting but forbidden zone of hindsight,' when analyzing the patentability of claims pursuant to that section. Measuring a claimed invention against the standard established by section 103 requires the oft-difficult but critical step of casting the mind back to the time of the invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then accepted wisdom in the field. Close adherence to this methodology is especially important in the case of less technologically complex inventions, where the very ease with which the invention can be understood may prompt one' to fall victim to the insidious effect of a hindsight syndrome wherein that which an inventor taught is used against its teacher."

"Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of a teaching or motivation to combine prior art references. . . .Combining prior art references without evidence of such a suggestion, teaching or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability - the essence of hindsight. . . ."

"[E]vidence of a suggestion, teaching or motivation to combine may flow from the prior art references themselves, the knowledge of one of ordinary skill in the art, or, in some cases from the nature of the problem to be solved, . . . although 'the suggestion [for the combination] more often comes from the teachings of the pertinent references.' The range of sources available, however, does not diminish the requirement for actual evidence. That is, the showing must be clear and particular. Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence'. . . . In addition to demonstrating the propriety of an obviousness analysis, particular factual findings regarding the suggestion, teaching, or motivation to combine serve a number of important purposes, including: (1) clear explication of the position adopted by the Examiner ...; (2) identification of the factual disputes;. . . (3) facilitation of the review on appeal."

Based on the foregoing, therefore, any rejection based on a combination of references must make particular findings regarding the locus of the suggestion, teaching or motivation to combine the prior art references. It is insufficient to limit an obviousness rejection to a discussion of the ways that the multiple prior art can be combined to read on the claimed invention. A mere reference-by-reference limitation analysis fails to demonstrate how the references teach or suggest their combination to yield the claimed invention. If one cannot discern in the rejection any finding that there was a suggestion, teaching, or motivation to combine the prior art references cited against the pending claims, the conclusion of obviousness as a matter of law cannot stand.

According to the Office Action, it would have been obvious to one or ordinary skill in the art at the time of the invention to "substitute the apoptotic inhibitor used by Cuevas et al. [FGF] with the Akt molecule taught by Datta et al in order to treat a myocardial infarction because both molecules are well-known in the art to function as inhibitors of apoptosis" (Office Action page 5). Based on this rationale for the obviousness rejection, one would necessarily conclude that one skilled in the art would have been motivated to substitute *any* apoptosis inhibitor for FGF for treating *any* disease than involved apoptotic cell death. The mere nexus that FGF is an apoptosis inhibitor is: (1) insufficient to motivate one skilled in the art to select Akt over other apoptosis inhibitors that were available at the time the invention was made; and (2) is insufficient to provide one skilled in the art with a reasonable expectation of success that the selected Akt would be useful for treating disorders mediated by apoptotic cell death in cardiomyocytes, skeletal myocytes and/or vascular endothelial cells. In summary, the generic basis offered in Office Action for rejecting the claimed invention lacks the particular findings of fact to support a prima facie showing of non-obviousness as required by the Dembiczak Court.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-4 under 35 U.S.C. §103(a) in view of the cited art.

Claim Objection:

Claim 5 is objected to as being dependent upon a rejected base claim. Applicants appreciate that the Examiner has found the claim 5 allowable over the prior art of record; however, Applicants have elected not to rewrite this claim in independent form in view of the above-presented arguments in favor of the patentability of the base claim.

Summary:

Applicants believe that each of the pending claims now is in condition for allowance. Applicants respectfully request that the Examiner telephone the undersigned attorney in the event that the claims are not found to be in condition for allowance.

If the Examiner has any questions and believes that a telephone conference with Applicant's attorney would prove helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned at (617) 720-3500 (extension 343).

Respectfully submitted,

WOLF, GREENFIELD & SACKS, P.C.

By _____
Elizabeth R. Plumer, Reg. No. 36,637
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, MA 02210-2211
Attorneys for Applicant(s)
Tel. no. (617) 720-3500

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